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(54) Method for improving flour dough.

(57) This invention relates to a method for improving the rheological properties of a flour dough which comprises combining flour, yeast, water and an effective amount of an enzyme preparation comprising sulfhydryl oxidase and glucose oxidase and mixing said ingredients to form a suitable baking dough. The invention results in stronger doughs with improved rheological properties as well as a final baked product with improved texture.

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Method for improving flour dough

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The "strength" or "weakness" of dough is an important aspect of baking. Flours with a low protein content are customarily characterized as "weak"; the gluten (the cohesive, extensible, rubbery mass which is formed by mixing flour and water) formed with weak flour will be very extensible under stress, but will not return to its original dimensions when the stress is removed. Flours with a high protein content are customarily characterized as "strong" and the gluten formed with strong flour will be less extensible than a weak flour, and stress which is applied during mixing will be restored without breakdown to a greater extent than a weak flour. Strong dough is generally preferred in most baking contexts because of the superior rheological and handling properties of the dough and the superior form and texture qualities of the final baked product made from the dough.

For example, stronger dough is generally more stable; the stability of dough is one of the most important (if not the most important) characteristics of baking dough.

American Association of Cereal Chemists Method 36-01A defines dough stability as "(a) the range of dough time over which a positive Response is obtained; and (b) that property of a rounded dough by which it resists flattening under its own weight over a course of time". Response is defined, by the same Method, as "the reaction of dough to a known and specific stimulus, substance or set of conditions, usually determined by baking it in comparison with a control".

Stable dough is particularly useful in large scale applications where it may be difficult to control all processing parameters; strong dough will exhibit a greater tolerance of, e.g. mixing time and proofing time, and still result in quality products. Less stable dough will exhibit less tolerance in this regard.

Bakers have long used dough "conditioners" to strengthen the dough. It is suggested that such conditioners, which consist primarily of non-specific oxidants such as bromates, peroxides, iodates and ascorbic acid, help form inter-protein bonds which strengthen the dough. However, non-specific oxidants have numerous drawbacks; in particular, they can have a negative effect on the organoleptic qualities of the final product and are relatively expensive in commercial quantities and, in the case of bromates, are not permitted in certain countries.

The use of enzymes as dough conditioners has been considered as an alternative to non-specific oxidants. In particular, glucose oxidase has been used - sometimes in combinations with other conditioners - to condition or "mature" flour. U.S. Patent No. 2,783,150 (Luther) discusses the treatment of flour with glucose oxidase with allegedly can be used to form an improved dough with better handling properties and a high quality final baked product. However, the effects of glucose oxidase are somewhat contradictory. Water absorption of the dough is increased but glucose oxidase, in some contexts, may actually impair dough rheology and has never been successfully used as a dough conditioner.

It has also been suggested that the enzyme sulfhydryl oxidase could be used to strengthen dough. Sulfhydryl oxidase ("SHX") catalyzes - in the presence of oxygen - the conversion of thiol compounds to their corresponding disulfides according to the equation:

$$2RSH + O_2 \rightarrow RSSR + H_2O_2$$

The role played by sulfur containing reactive groups in wheat proteins has not been fully defined but it is suggested that the reaction of free sulfhydryl groups to form disulfide bonds has an important role in the mixing and strength of dough. In particular, if disulfide bonds are formed between two protein chains, the resulting cross-linking of chains could strengthen the dough. Hence, SHX could be expected to strengthen dough by catalyzing the reaction of free sulfhydryl groups into inter-protein disulfide bonds.

However, Kaufmann et al., Cereal Chemistry 64:3 (1987), evaluated bovine SHX's ability to strengthen wheat dough and concluded that it did not have any strengthening effect. The baking tests reported by Kaufmann et al. did not show any "noticeable" effect of SHX on loaf volume, and mixograph studies on SHX treated dough which did not show any "noticeable" effect on the time to reach a mixing peak or the extent of dough breakdown. Kaufmann et al. also evaluated the effect of SHX on flour/buffer suspensions and concluded that SHX did not show any effect on the free-SH groups of flour. Kaufmann et al. state that - for a number of possible reasons - SHX was not able to catalyze formation of disulfide bonds in the systems t sted.

It has now been discov red, however, that inclusion of an enzyme preparation which comprises glucose oxidase and microbial SHX into a flour, water and yeast mix appreciably and significantly strengthens the resulting dough; the dough exhibits improved rheological qualities, and in particular, demonstrates increased stability. The final, baked product mad from such dough also exhibits improved form and texture qualities.

The present invention contemplates a method for improving rheological properties of flour dough by combining therewith an effective amount of sulfhydryl oxidase and glucose oxidas . In a preferred embodiment, a flour dough is formed by mixing flour, preferably wheat flour, together with water, yeast and an enzyme preparation comprising glucose oxidase and SHX to form a dough. The resulting dough exhibits improved strength and rheological properties. The enzyme preparation may, in addition, contain catalase. The enzyme preparation is preferably elaborated from microbial sources, including Aspergillus niger.

Preferably the enzyme preparation contains about 35 and about 800 Units (as hereinafter defined) of SHX per kilogram of flour, with a concentration of about 80 Units of SHX being particularly preferred. The present invention also contemplates a dough conditioner wherein the ratio of SHX/glucose oxidase (based on units of enzyme present) is in the range of about 0.003 to about 10. A ratio of about .35 is particularly preferred.

The method of the present invention can also be used in combination with a non-specific oxidant such as ascorbic acid.

In order to demonstrate the effectiveness of a glucose oxidase-sulfhydryl oxidase enzyme preparation as a dough conditioner, varying levels of glucose oxidase and sulfhydryl oxidase were added to flour, water, yeast and salt, and mixed to make a suitable baking dough. The examples set forth in Tables I, II and III were made with a wheat flour (consisting of 95 % Finnish wheat and 5 % wheat from sources outside Finland). The flour used to make the doughs set forth in Table III were treated with ascorbic acid, a non-specific oxidant. For all samples set forth in Tables I and II, 1200 g of wheat was combined with Bakers yeast, 38 g, salt, 20 g, and water, 28 g and varying amounts of an enzyme preparation purified from Aspergillus niger cells by cell filtration and having the following activity levels:

SHX	7.7 U/mg
glucose oxidase	21.9 U/mg
catalase	0.17 U/mg

The term "Units" as used herein and in the appended claims means as follows:

SHX Unit: one sulfhydryl oxidase Unit is the amount of enzyme required to deplete 1 micromole of O_2 per minute from an assay mixture containing 8 millimoles GSH and 40 millimoles sodium acetate at pH 5.5 and a temperature of 25° C. (Young J. and Nimmo I., Biochem. J. (1972), 130:33.).

Glucose Oxidase Unit: 3 Units of glucose oxidase produce 1 ml of 0.05-N gluconic acid. (Scott D., J.Agr.Food Chem. (1953), 1:727.)

Catalase Unit: one catalase Unit decomposes 265 mg of hydrogen peroxide under the assay conditions of 25° C., 1.5 % hydrogen peroxide and a pH of 7.0 when reacted to exhaustion. (Scott D. and Hammer F., Enzymologia (1960), 22:194.)

The flour, water, yeast, salt and enzyme preparation were mixed in a high speed dough mixer for 71 seconds (Stephan) to develop a suitable baking dough. A control dough - without the addition of any enzyme preparation - was also prepared. The effect of the enzyme preparation on the dough was measured by following standard methods according to the International Association of Cereal Chemistry (ICC) and the American Association of Cereal Chemistry (AACC): amylograph (ICC 126), farinograph (AACC 54-21) and extensigraph (AACC 54-10).

The amylograph, farinograph and extensigraph are indirect methods - used by bakers worldwide - to measure the rheological properties of dough. The amylograph determines the viscosity changes of flour-water suspensions during increasing temperatures (which are increased at a rate of 1.5° C./min.). At approximately 65° C. the mixture begins to gelatinize and typically reaches its maximum viscosity at about 80° C. depending on the characteristics of the flour. The resulting amylograph curve illustrates the viscosity changes of flour; generally, flour with good baking properties exhibits higher viscosity at higher temperature for its maximum gelatinizing point.

AACC Method 22-10 defines the amylograph as follows: "the amylograph is a recording viscometer that may be used primarily to determine effect of alpha-amylase on viscosity of flour as a function of temperature. The high viscosity of the starch gel is counteracted by the action of alpha-amylase, which

liquefies starch granules during heating of slurry. The amylograph value, or malt index, provides information on probable effect of malt alpha-amylase during baking process".

The extensigraph measures such properties as the dough's ability to retain gas evolved by yeast and the ability to withstand proofing. In effect, the extensograph measures the relative strength of a particular dough. A strong dough will exhibit a higher and longer extensograph curve than a weak dough.

AACC Method 54-10 defines the extensigraph as follows: "the extensigraph records a load-extension curve for test piece of dough stretched until it breaks. Characteristics of load-extension curves or extensigrams are used to assess general quality of flour and its responses to improving agents".

The farinograph method determines the water intake of a particular flour and the mixing tolerance of the resulting dough. Better baking flours, and dough, will exhibit higher farinograph values. If a particular flour shows relatively high water intake, and the mixing tolerance of the resulting dough is good, the farinograph curve shows retention of most if not all of the initial height over time. The machinability and baking quality of such a dough is likely to be excellent.

AACC Method 54-21 defines the farinograph as follows: "the farinograph measures and records resistance of a dough to mixing. It is used to evaluate absorption of flours and to determining stability and other characteristics of doughs during mixing".

Baking conditions used for baking bread from the dough prepared as above were as follows:

oven: normal hearth oven (Dahlen) w/10
seconds steaming
flour time: 30 minutes
final proofing: 30 minutes/37° C./75 % humidity
baking time: 25 minutes at 220° C.
cooling time: 1 hour/20° C.

TABLE I

Comparison of Rheological Properties of Doughs					
ENZYME ADDITION	Dough Sample				
	1	2	3	4	5
SHX Units/kg flour	-	38	77	154	770
GO Units/kg flour	-	110	219	438	2190
CAT Units/kg flour	-	2	4	7	40
SHX/GO Unit ratio	-	0.34	0.35	0.35	0.35
GO/CAT Unit ratio	-	55	55	55	55
RESULTS					
Farinogram Water absorption %	62	62	62	62	62
Development of the dough/(measured in minutes)	2	3	3.5	2	2
Stability/(measured in minutes)	3	12	23	19	2
Softening/(after?) 12 min Brabender Units (B.u.)	50	30	10	10	120
Resistance to Extens. Brabender Units (B.u.)	420	450	580	610	980
Extension,mm Brabender Units (B.u.)	180	165	143	139	86
Ratio resistance/extens B.u./mm	2.4	2.7	4.1	4.4	11
Maximum extension (B.u.)	620	640	760	720	980

Table I, above sets forth rheological properties for four doughs, a control dough (dough #1) and doughs conditioned with the enzyme preparations having different levels of SHX, glucose oxidase and catalase (doughs #2, #3, #4 and #5). The data demonstrates that dough mixed with an enzyme preparation containing glucose oxidase and SHX exhibit significantly improved rheological properties when compared with the control dough. In particular, dough#3 (23 minutes) and dough#4 (19 minutes) exhibit dramatic increases in stability compared to the control dough (3 minutes). The improved stability of the doughs treated with the enzyme preparation indicates that such doughs will exhibit better handling and machinability properties. The analysis data for the doughs treated with the enzyme preparation also suggests that these doughs were significantly strengthened. The resistance to extension, the maximum extension and the ratio of resistance to extension all indicate that the treated doughs were significantly strengthened.

As noted, doughs with improved stability and strength generally also result in final baked products with

improved qualities. Baking tests confirmed that doughs treated with the method of the present invention provided superior final products.

TABLE II

Results of Baking Tests			
	Loaf Sample		
	1	2	3
Dough sample (Table I)	1 (control)	3	4
Dough consistency			
After mixing (B.u.)	325	315	320
After floor time (B.u.)	285	290	300
Loaf weight (g)	370	370	370
Loaf height (mm)	76	79	76
Loaf width (mm)	172	171	173
Loaf H/W ratio	0.44	0.46	0.44
Loaf volume (ml)	1230	1340	1290
Loaf Spec.Vol. (ml/kg)	3310	3620	3490
Loaf moisture (%)	45.2	45.0	45.0

Table II sets forth baking results for loafs baked from doughs # 1 (control), # 3 and # 4 referred to in Table I. The control dough (loaf # 1) was not treated with any enzyme preparations and dough samples # 3 and # 4 (loaf samples # 2 and # 3 respectively) were treated with the enzyme preparation as set forth in Table I. The data from the baking tests set forth in Table II demonstrates that - compared to the control dough - doughs treated with the SHX/glucose oxidase enzyme preparation exhibited improved size and texture. In particular, loaf sample # 2 (baked with dough sample # 3) exhibited higher loaf volume (1340 ml. versus 1230 ml), higher specific volume (3620 ml/kg versus 3310 ml/kg) than the control sample. Thes height and width values demonstrate that loaf samples # 2 and # 3 were rounder and more symmetrical in shape, evidence of greater dough strength. In addition, the porosity of these loafs were more uniform meaning that the pores are the same size both near the crust and the center of the loafs.

Organoleptic comparison of the three loaf samples indicated that loaf samples # 2 and # 3 demonstrated improved texture properties compared to the control.

The baking results suggest that the present invention will help bakers achieve a larger loaf volume. In the commercial context this means that bakers could use wheat with a lower protein content, which is cheaper, to achieve the desired loaf size and/or can utilize a smaller dough plug to achieve the desired loaf size; both possibilites could potentially result in substantial savings in material costs to the baker.

In order to determine the effect of varying levels of SHX and glucose oxidase in enzyme preparations used to treat baking doughs, the following enzyme samples were developed:

Enzyme Sample A (prepared from A. niger cells)	
Activities:	
GO	1.0 U/mg
SHX	11.5 U/mg
CAT	–
Enzyme Sample B (prepared from A. niger cells)	
Activities:	
GO	129 U/mg
SHX	0.4 U/mg
CAT	0.2 U/mg
Enzyme Sample C (prepared from A. niger cells)	
Activities:	
GO	122 U/mg
SHX	0.8 U/mg
CAT	2.0 U/mg

Tables III and IV below set forth data regarding the rheological properties of doughs prepared with Enzyme Samples A, B and C as well as a control dough.

TABLE III

Rheological Properties of Dough Samples - Various Enzyme Levels											
Enzyme Sample	Activities			Water Absorp %	Dough Devel min	Dough Stabil min	Dough Soft B.u.	Resist B.u.	Ext min	Res/Ext ratio	Max B.u.
	GO	SHX	Catalase								
Control				62	1.5	2.0	50	420	180	2.4	620
Enzyme Sample A											
1.	4	38		62	1.5	2.5	50	470	176	2.7	640
2.	8	77		62	1.5	2.0	60	470	174	2.7	640
Enzyme Sample B											
1.	645	2	1	62	2	3.5	20	640	125	5.1	740
2.	1290	4	2	62	2	15	10	640	126	5.1	760
3.	2580	8	4	62	2	14.5	20	660	125	5.3	760
4.	12900	40	20	62	2	2.5	120	980	95	10	980
Enzyme Sample C											
1.	1290	8.0	20	62	2	3	30	720	129	5.6	900

The doughs set forth in Table III w r prepared with "weak" Finnish wheat flour that had been treated

with ascorbic acid (a non-specific oxidant) and preparation as described above. Although this data suggests, as indicated by the prior art, that glucose oxidase alone can have a conditioning effect, relatively large (and uneconomical) quantities of glucose oxidase are required to achi v appreciable strengthening. The data also suggests that a glucos oxidase/SHX combination is the most efficient and economical preparation for dough conditioning.

TABLE IV

Rheological Properties of Dough Samples											
Enzyme Preparations	Activities			Water Absorp %	Dough Devel min	Dough Stabil min	Dough Soft B.u.	Resist B.u.	Ext min	Res/Ext ratio	Max B.u.
	GO	SHX	Catalase								
Amer.	-	-	-	60	2.5	10	40	330	261	1.3	620
Bromated	110	38	2	60	2	15	0	400	210	1.9	720
Flour	219	77	4	60	2	15	-0	670	175	3.8	900
(strong)	440	154	7	60	5	13	0	735	168	4.4	860
Amer.	-	-	-	61	2.5	8	40	340	224	1.5	640
Unbromated	110	38	2	61	2.5	12	20	390	188	2.1	580
Flour	219	77	4	61	2	15	0	525	178	3.0	720
(strong)	440	154	7	61	5	12	0	610	182	3.4	720
Amer.	-	-	-	57	1.5	4	90	280	172	1.6	390
(Weak)	110	38	2	57	1.5	3	100	370	149	2.5	440
+ SHX	219	77	4	57	1.5	3.5	70	430	137	3.1	480
(x2620)	440	154	7	57	1.5	4	60	515	140	3.7	560

Table IV sets forth data for doughs prepared using different US flours, both strong and weak. Compared to the control samples, doughs prepared with these flours that were treated with an SHX/glucose oxidase enzyme preparation generally demonstrated increased stability and strength, as indicated by the resistance and extension data. The effect was greater in the case of the "strong" flours (bromated and unbromated) than for the unbromated "weak" flours.

Table V set below sets forth data regarding rheological properties of doughs prepared with Finnish rye meal. A control dough with no enzyme treatment (sample # 1) and dough samples with varying levels of SHX and glucose oxidase were prepared.

TABLE V: Rheological Properties of Rye Dough* Samples

Enzyme Preparations	Activities			Water Absorp %	Dough Devel min	Dough Stabil min	Dough Soft B.u.	Au/'C
	GO	SHX	Catalase					
0 - Control				75	3.5	3	20	
1.	110	38	2	73	3	>12	0	
2.	219	77	4	73	3	>12	0	
3.	440	154	7	74	3	>12	+10	
4.	645	2	1	74	3.5	>12	0	
5.	1290	4	2	74	3.5	>12	+10	
6.	2580	8	4	75	3.5	>12	+10	
7.	12900	40	20	74	3.5	6	20	

*Analysis of the rye meal samples:

Ash content 1.74 %

Falling number 125

Amylogram 260 B.U. at 66°C

The data in Table V shows that an enzyme preparation with SHX/GO acts as a dough conditioner; the most efficient - and cost effective - preparations (samples 1-3) will probably be more effective in large scale contexts.

The results of these experiments demonstrate that use of an enzyme preparation containing SHX and glucose oxidase appreciably and significantly improves the rheological properties of dough. The effect of

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EUROPEAN SEARCH REPORT

Application Number

EP 88 12 0669

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,X	US-A-2 783 150 (H.G. LUTHER) * Column 1, line 40 - column 2, line 11; column 2, line 45 - column 3, line 5; claim 1 *	1,2,5,7,8	A 21 D 8/04
A	BROT UND GEBÄCK, vol. 9, no. 6, June 1955, pages 101-103; P. MALTHA: "Weitere Untersuchungen über die Backverbesserung durch 1-Ascorbinsäure" * Whole document *	1,5,7,8	
A	CHEMICAL ABSTRACTS, vol. 71, no. 11, 24th November 1969, pages 225,226, no. 100605k, Columbus, Ohio, US; M.V. POLYAK et al.: "Glucose oxidase as a conditioner in bread baking", & FERMENTY MED., PISHCH. PROM. SEL. KHOZ. 1968, 155-7 * Abstract *	1,5,7,8	
D,A	CEREAL CHEMISTRY, vol. 64, no. 3, May-June 1987, pages 172-176, St. Paul, MN, US; S.P. KAUFMAN et al.: "Evaluation of sulfhydryl oxidase as astrengthening agent for wheat flour dough" * Abstract *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 21 D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	09-03-1989	GROENENDIJK M.S.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
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